ORIGINAL

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07D 309/28 — A1

(11) International Publication Number: WO 95/00503

(43) International Publication Date: 5 January 1995 (05.01.95)

(21) International Application Number: PCT/EP94/01940

(22) International Filing Date: 15 June 1994 (15.06.94)

(30) Priority Data:
9312531.8 17 June 1993 (17.06.93) GB

(71) Applicant (*for all designated States except US*): GLAXO GROUP LIMITED [GB/GB]; Glaxo House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): SEARLE, Andrew, David [GB/GB]; Glaxo Research and Development Limited, Greenford Road, Greenford, Middlesex UB6 0HE (GB). WILLIAMSON, Christopher [GB/GB]; Glaxo Research and Development Limited, Greenford Road, Greenford, Middlesex UB6 0HE (GB).

(74) Agents: QUILLIN, Helen, Kaye et al.; Glaxo Holdings plc, Glaxo House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

09/555,442

(54) Title: PROCESS FOR THE PREPARATION OF N-ACETYL NEURAMINIC ACID DERIVATIVES

(57) Abstract

A method for the preparation of 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid is described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

PROCESS FOR THE PREPARATION OF N-ACETYL NEURAMINIC ACID DERIVATIVES

The present invention relates to a process for the preparation of derivatives of N-acetyl neuraminic acid. More particularly the invention relates to a process for the preparation of 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (the 4-amino analogue of DANA; also known as 5-(acetylamino)-4-amino-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enonic acid).

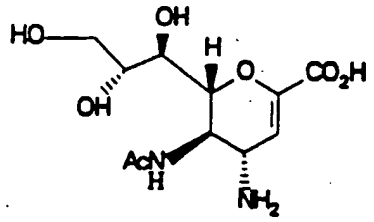
Schreiner et. al. Ann. Chem 1991, 129-134 describe the preparation of the 4-amino analogue of DANA from the peracetylated methyl ester of sialic acid (peracetyl NANA methyl ester) by the route shown in Scheme 1.

PCT/AU91/00161 (publication no. WO91/16320) describes the preparation of a number of derivatives of 5-acetamido 2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (2,3-dideoxy-2,3-didehydro-N- acetyl-neuraminic acid; DANA) including the 4-amino analogue of DANA from the peracetylated methyl ester of DANA by a method similar to that of Schreiner et. al. with the exception that the peracetylated compound (3a) was reduced prior to deacetylation. The method is shown in Scheme 2.

Our copending application no. PCT/EP92/02904,publication no. WO 93/12105, published 24 June 1993, describes a process for the preparation of 4-amino DANA from the corresponding peracetyl-4-azido analogue by catalytic hydrogenation using gaseous hydrogen.

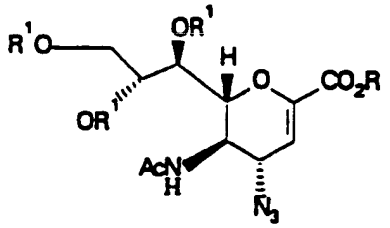
We have now found that the yield and purity of 4-amino DANA can be improved by modification of the conversion of peracetyl 4-azido DANA to 4-amino DANA described in PCT/EP92/02904.

The invention thus provides a method for the preparation of the compound of formula (I)



(I)

which comprises catalytic hydrogenolysis of a compound of formula (II)



(II)

(wherein R is H or a C_{1-4}alkyl group and R^1 is H or a hydroxyl protecting group for example an acyl group such as acetyl) in aqueous formic acid followed, where necessary, by hydrolysis.

By aqueous formic acid is meant a solution of formic acid in water or a mixture of water and any compatible organic solvent miscible with water. Preferably the solvent is water. Formic acid is conveniently present in an amount of 1-4 molar equivalents, for example about 2 molar equivalents of the compound of formula (II).

The formic acid acts as the source of hydrogen for the hydrogenolysis. It will be appreciated by those skilled in the art that the hydrogenolysis of the compound of formula (I) results in the liberation of nitrogen gas; it is thus advantageous to employ formic acid rather than hydrogen gas as the source of hydrogen.

The catalytic reduction may be effected with any suitable catalyst. In one preferred embodiment the catalyst is a palladium catalyst but in particular a poisoned palladium catalyst. A preferred poisoned palladium catalyst is a Pd catalyst

poisoned with lead for example a Lindlar Catalyst. A particularly preferred catalyst comprises 5% palladium on a suitable support such as barium sulphate or, preferably, calcium carbonate,and 3 to 7% lead by weight of catalyst, such as 4 to 6% lead, more preferably 5% lead.

In an alternative preferred embodiment the catalyst is an unpoisoned catalyst, in particular, a palladium catalyst, for example palladium on charcoal.

The reduction is conveniently carried out at 0-50°C, preferably at 20-30°C .

In a preferred aspect of the invention the compound of formula (II) is deprotected, that is to say that R and R^1 are both hydrogen. The compound of formula (II) wherein R and R^1 are both hydrogen may be obtained by hydrolysis of the corresponding compound of formula (II) wherein R is a C_{1-4} alkyl group and R^1 is a hydroxyl protecting group such as acetyl. The hydrolysis may be effected with any suitable inorganic or organic base. Preferably an organic base such as triethylamine or, particularly, 1,8-diazabicylo [5.4.0]undec-7-ene (DBU) is employed. Water or a mixture of water and any compatible organic solvent miscible with water is conveniently employed as the solvent. Preferably water is used. The reaction is conveniently carried out at ambient temperature. Conveniently at least 5 molar equivalents of base will be employed; alternatively a catalytic quantity of base may be employed where trans-esterification is used by incorporation of an alcohol, eg methanol, in the solvent mixture.

To facilitate isolation of the compound of formula (I), the pH of the reaction mixture is preferably optimised. Optimal pH for the isolation of the compound of formula (I) is in the range of 3 to 9, preferably 5 to 8, more preferably about 6.5. Adjustment to optimal pH may suitably be effected by addition of an acid, such as an organic acid, for example formic acid, or a base, such as an organic base, for example 1,8-diazabicyclo[5.4.0]undec-7-enem (DBU), as appropriate.

The compound of formula (I) may be isolated by any convenient method known in the art. However, the compound of formula (I) is preferably obtained by crystallisation. By this means 4-amino DANA is readily obtained substantially free of impurities.

Crystallisation can be induced by treating an aqueous solution of 4-amino DANA with an antisolvent, for example *iso*-propanol, at a temperature of from 10-80 °C, for example 25-70°C such as about 60°C.

The 4-amino analogue of DANA is a potent inhibitor of influenza virus both in vitro and in vivo and is thus useful in the treatment of viral infections such as influenza. (see for example WO91/16320).

The 4-amino analogue of DANA is also of use as an intermediate in the synthesis of other DANA derivatives which are inhibitors of influenza virus (see for example WO91/16320).

The invention is illustrated by the following non-limiting examples. All temperatures are in °C.

Example 1

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate as its monohydrate, (20g) was suspended in water (40ml) and chilled with stirring to 10°. 1,8-Diazabicyclo[5.4.0]undec-7-ene (31.5ml) was added over 20 minutes. The mixture was then stirred at 20°C for 1.5h, giving a clear solution.

Lindlar catalyst (1g), then formic acid (3.2ml) were added and the mixture stirred at 20° for 16h. Filter aid (1g) was added, then reaction mixture filtered to remove the catalyst. The spent catalyst bed was washed with water (3 x 0.67ml).

The filtrate and washings were combined and formic acid was added till a pH of 6.5 was reached. The resulting solution was heated to 60° and the product was crystallised by adding *iso*-propyl alcohol (400ml) in portions.

The resulting crystalline slurry was cooled to 5° overnight, then filtered, and the product washed with *iso*-propyl alcohol (2 x 40ml), then dried *in vacuo* at 40° for 16h, to give the 4-amino analogue of DANA (12.25g) as a trihydrate.

N.M.R. (D_2O)5.62 (1H,d,2); 4.40-4.25(2H,m);

	4.18 (1H,m);4.05-3.50 (4H,m); 2.08 (3H,s)
I.R. (Nujol)	3526, 3477, 3368, 3179 (HO,NH); 1675 (CO, amide), 1601cm^{-1} (CO, CO_2H)

Example 2

A solution of Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate as its monohydrate, (2g) in water (6ml) and methanol (14ml) was treated with triethylamine (1.17ml) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.89ml). After stirring the resulting solution for 1.2h at 20°, 10% palladium on activated carbon catalyst (0.2g) and formic acid (0.32ml) were added. The mixture was stirred under nitrogen at 20° for 18h, then filter aid (0.2g) added.

The reaction mixture was filtered to remove the catalyst, then the spent catalyst bed was washed with water (2 x 4ml). The filtrate and washings were combined and concentrated to 16ml *in vacuo*. The resulting solution was heated to 60° and the product crystallised by adding *iso*-propyl alcohol (80ml) in portions.

The resulting crystalline slurry was cooled to ambient temperature, then filtered, and the product washed with *iso*-propyl alcohol (2 x 4ml), then dried *in vacuo* at 40°, to give the 4-amino analogue of DANA (0.63g), identical to the product of Example 1.

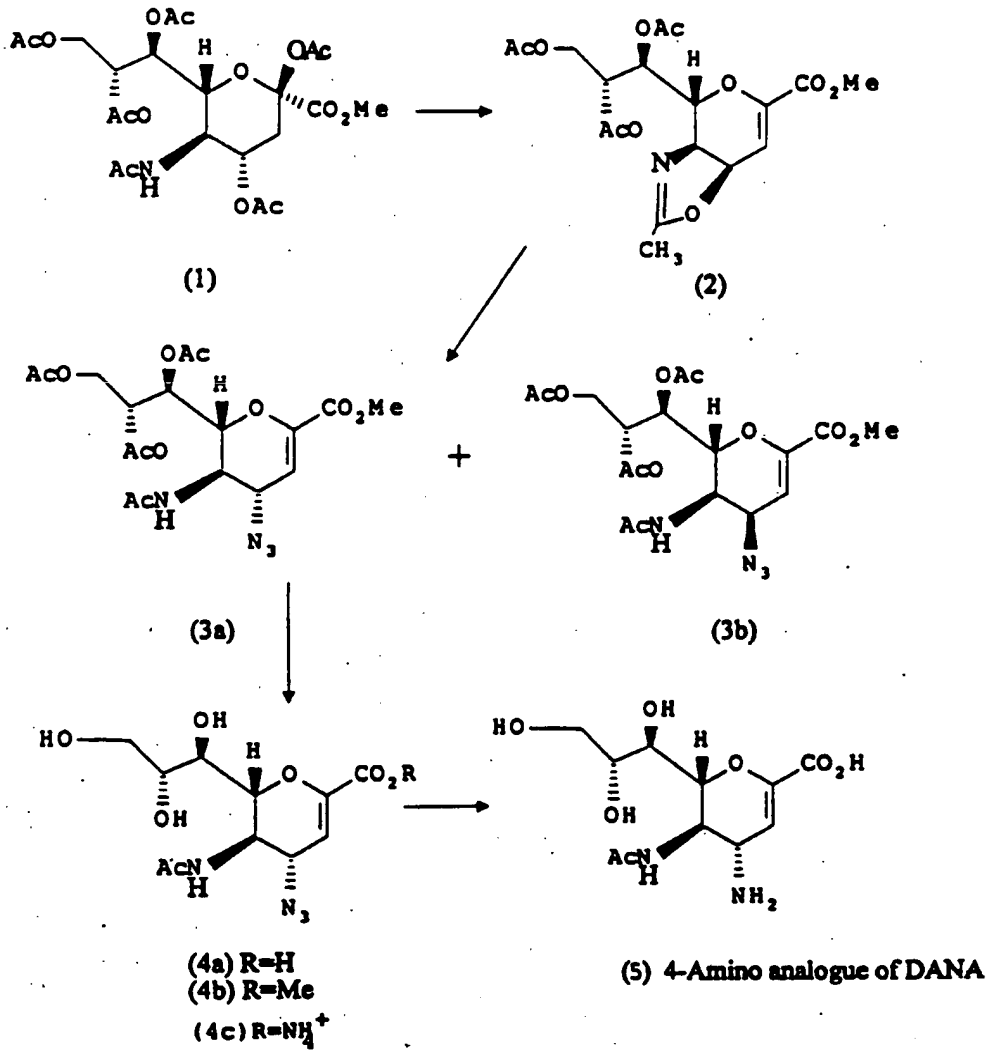
Example 3

A solution of Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate as its monohydrate, (100g) in water (150ml) at about 10° was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (158ml) and stirred at about 20° for *ca.* 1.5h, giving a solution. Lindlar catalyst (5g) comprising 5% palladium on calcium carbonate and 5% lead was added, followed by formic acid (15.9ml) whilst the temperature was kept below 30°. The mixture was maintained at 25-30° for approximately 16h. The catalyst was removed by filtration through a prepared filter-bed, which was then washed with water (2x50ml).

The combined filtrate and washings were adjusted to pH6.5 by addition of formic

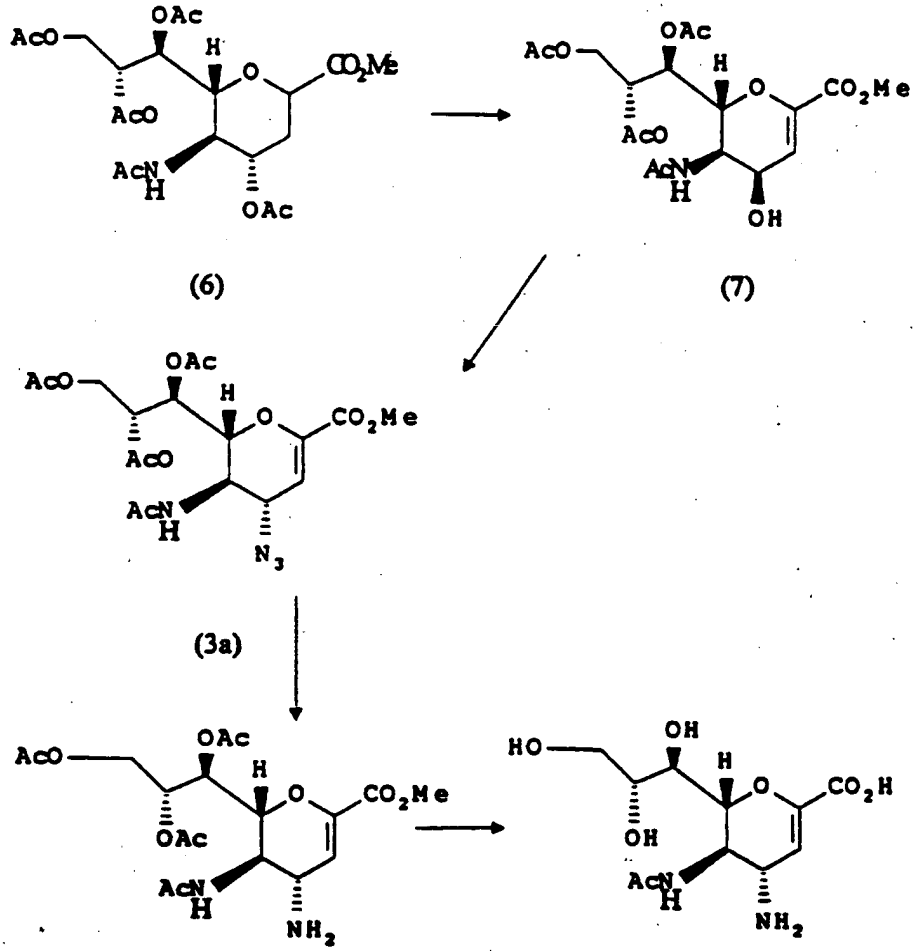
acid (10ml). The solution was stirred for 30min, then re-acidified to pH6.5 by addition of a further few drops of formic acid. The solution was warmed to about 60° and diluted with *iso*propanol (800ml), then allowed to crystallise. The mixture was reheated to 60° and further diluted with *iso*propanol (1200ml). The resulting suspension was stirred for approximately 16h, then cooled to about 5°. The solid was isolated by filtration, washed with *iso*propanol (2x200ml) and vacuum dried at 40° to give the 4-amino analogue of DANA (62.4g), identical to the product of Example 1.

Scheme 1


AcO
OAc
H
O
OAc
CO2Me
AcO
AcN
H
OAc
(1)
CH3
(2)
N3
(3a)
(3b)
HO
OH
CO2R
(4a) R=H
(4b) R=Me
(4c) R=NH4+
CO2H
NH2
(5) 4-Amino analogue of DANA

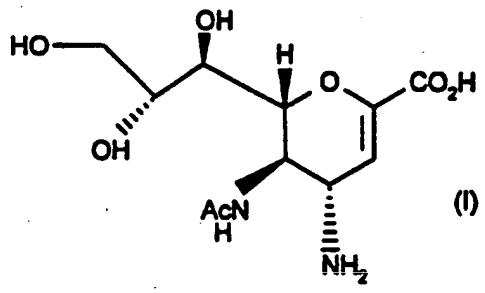
145

Scheme 2


AcO
OAc
H
O
CO2Me
AcO
AcN
H
OAc
(6)
OH
(7)
N3
(3a)
NH2
(8)
HO
(5) 4-Amino analogue of DANA

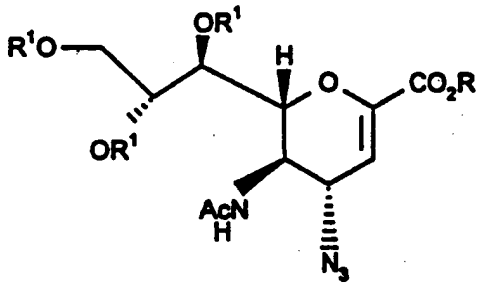
Claims

1. A process for the preparation of a compound of formula (I)



(I)

which comprises catalytic hydrogenolysis of a compound of formula (II)



(II)

wherein R is H or C_{1-4}alkyl and R^1 is H or a hydroxyl protecting group, in aqueous formic acid.

2. A process as claimed in Claim 1 wherein the catalyst is a palladium catalyst.

3. A process as claimed in Claim 1 or Claim 2 wherein the catalyst is a poisoned palladium catalyst.

4. A process as claimed in any one of Claims 1 to 3 wherein the catalyst is a palladium catalyst poisoned with lead.

5. A process as claimed in any one of Claims 1 to 4 wherein the catalyst is a Lindlar catalyst.

6. A process as claimed in any one of Claims 1 to 5 wherein the catalyst comprises 5% palladium on a support and 3 to 7% lead.

7. A process as claimed in Claim 6 wherein the support is calcium carbonate.

8. A process as claimed in Claim 6 or Claim 7 wherein the lead content is 5%.

9. A process as claimed in any one of Claims 1 to 8 wherein the formic acid is present in an amount of 1-4 molar equivalents of the compound of formula (II).

10. A process as claimed in Claim 9 wherein the formic acid is present in an amount of about 2 molar equivalents of the compound of formula (II).

11. A process as claimed in any one of Claims 1 to 10 which is carried out at a temperature of 0 to 50°C.

12. A process as claimed in any one of Claims 1 to 11 wherein in the compound of formula (II) at least one of R and R^1 is not hydrogen and wherein the product of reduction is subsequently hydrolysed.

13. A process as claimed in Claim 12 wherein the hydrolysis is effected in aqueous medium.

14. A process as claimed in Claim 12 or Claim 13 wherein the hydrolysis is effected with a base selected from triethylamine and 1, 8-diazabicyclo [5.4.0] undec - 7 - ene.

15. A process as claimed in any one of Claims 1 to 14 wherein the pH of the reaction mixture is adjusted to within the range of 3 to 9 prior to isolation of the compound of formula (I).

16. A process as claimed in claim 15 wherein the pH is adjusted to about 6.5.

INTERNATIONAL SEARCH REPORT

Intern. ional Application No
PCT/EP 94/01940

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07D309/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,91 16320 (BIOTA) 31 October 1991 cited in the application see page 21 - page 22; claims 16-19	1
P,X	WO,A,93 12105 (GLAXO GROUP) 24 June 1993 see claims 10-20	1-13

☐ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 October 1994	19. 10. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Francois, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ...nal Application No
PCT/EP 94/01940

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9116320	31-10-91	AU-A-	7533891	12-12-91
		AU-A-	7759091	11-11-91
		CN-A-	1057260	25-12-91
		EP-A-	0526543	10-02-93
		HU-A-	61989	29-03-93
		OA-A-	9679	15-05-93
WO-A-9312105	24-06-93	AU-B-	3158893	19-07-93